IS 548-2-9 (1988): Methods of sampling and test for oils and fats, Part II: Purity tests, Section 9: Test for presence of Karanja (Pungam) oils in other oils [FAD 13: Oils and Oilseeds]

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IS : 548 ( Part 2/Sec 9 ) - 1988
(Reaffirmed 2000)

*Indian Standard*

# METHODS OF SAMPLING AND TEST FOR OILS AND FATS

## PART 2 PURITY TESTS

### Section 9 Test for Presence of *Karanja* ( *Pungam* ) Oil in Other Oils

*( Fourth Revision )*

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# *Indian Standard*

# METHODS OF SAMPLING AND TEST FOR OILS AND FATS

## PART 2 PURITY TESTS

### Section 9 Test for Presence of ( *Karanja Pungam* ) Oil in Other Oils

## *( Fourth Revision )*

## 0. FOREWORD

**0.1** This Indian Standard ( Part 2/Sec 9 ) ( Fourth Revision ) was adopted by the Bureau of Indian Standards on 30 May 1988, after the draft finalized by the Oils and Oil Seeds Sectional Committee had been approved by the Chemical Division Council and Agricultural and Food Products Division Council.

**0.2** This standard was first published in 1954 and subsequently revised in 1964 as Part 1 and it covered methods of sampling, physical, chemical and qualitative tests.

**0.2.1** In view of periodical review of qualitative tests for detection of adulteration in oils and fats, it was decided to cover such tests in Part 2 of this standard and IS : 548 ( Part 2 )-1976* was accordingly published.

**0.2.2** It was felt that additional purity tests and the existing test methods when revised should be covered in the form of separate test methods and not added as amendments to the standard ( that is, Part 2 ) since it would create confusion. The tests covered under various sections of IS : 548 ( Part 2 )-1976* are as follows:

Section 6 Test for the presence of sesame oil ( modified Baudouin test )

Section 7 Test for the presence of cottonseed oil ( Halphen test )

Section 8 Test for the presence of linseed oil ( Hexabromide test )

Section 9 Test for the presence of *Karanja* ( *Pungam* ) oil and other oils containing phenolic substances

Section 10 Test for the presence of argemone oil by paper chromatographic method

Section 11 Test for the presence of hydrocyanic acid

Section 12 Test for the presence of mineral oil

Section 13 Test for the presence of groundnut oil [ Bellier turbidity temperature test ( Acetic acid method ) ]

Section 14 Test for the presence of *Kusum* oil and other oils containing cyanogenic compounds

Section 15 Test for the presence of castor oil

Section 16 Test for the presence of *Neem* oil

Section 17 Test for the presence of other oils in castor oil

Section 18 Test for the presence of animal fat in vegetable oils ( phytosterol acetate melting point test)

Section 19 Test for the presence of oil soluble colours in oils and fats

**0.3** Section 9 of IS : 548 ( Part 2 )-1976* has now been brought out as a separate standard after its revision. In the revised standard, two methods of tests have been prescribed for detection of *Karanja* oil in other oils. The earlier prescribed method was not very sensitive and, therefore, an additional TLC method which is more sensitive, has been specified in the revised standard. Subsequently, Section 9 of IS : 548 ( Part 2 )-1976* is being deleted through an amendment.

**0.4** *Karanja* **or** *Pungam* **( Pongamia Glabra, Pongamia Pinnata )**—Oil is a commercial non-edible oil. It is usually cheaper than many other vegetable oils and can perhaps be used for adulterating other dark-coloured and odoriferous oils in raw form and light coloured oils after refining.

**0.5** In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960†.

*Method of sampling and test for oils and fats: Part 2 Purity tests.

*Methods of sampling and test for oils and fats: Part 2 Purity tests.

†Rules for rounding off numerical values ( *revised* ).

## 1. SCOPE

**1.1** This standard ( Part 2/Sec 9 ) prescribes the methods of test for detection of *Karanja* ( *Pungam* ) oil in other oils.

## 2. GENERAL

**2.0** Two methods have been prescribed, namely, antimony trichloride and thin layer chromatography ( TLC ) method. In case of any dispute, the TLC method shall be the referee method.

## 3. METHOD I ( ANTIMONY TRICHLORIDE METHOD )

**3.0 General** — The non-saponifiable component of this oil reacts with antimony trichloride to form yellow to orange coloured complexes.

### 3.1 Reagent

**3.1.1** *Antimony Trichloride Solution* — 20 percent ( *m/v* ) in chloroform. The reagent is prepared by weighing antimony trichloride crystals, adding to chloroform and shaking for a few minutes till the crystals dissolve.

**3.2 Procedure** — Take one drop of the oil sample in a small test tube or in a depression of porcelain tile. Add 1 to 2 ml of antimony trichloride reagent. An immediate characteristic canary yellow to orange yellow colour shows the presence of *Karanja* ( *Pungam* ) oil in the sample.

**3.3 Sensitivity** — This test is sensitive to the extent of 3 percent of *Karanja (Pungam )* oil in other oils.

## 4. METHOD II ( THIN LAYER CHROMATOGRAPHY METHOD )

**4.0** It is a sensitive and specific method for detection of adulteration or contamination with *Karanja* oil, either raw or refined with alkali, acid, alcohol or alcoholic alkali and bleached. The method is based on the separation of minor constituents present in *Karanja* oil by thin layer chromatography ( TLC ) and their visualization in ultraviolet light.

**4.1 Outline of the Method** — A solution of the oil sample to be tested and of the pure reference oil are applied on micro slide or regular glass plate coated with silica gel and developed with a solvent mixture. In ultraviolet light, an additional flourescent spot, having a Rt value of about 0·27, is seen when *Karanja* oil is present.

### 4.2 Material

**4.2.1** *Adsorbent* — Silica gel containing a binder ( silica gel 'G' for use in TLC ).

**4.2.2** *Solvents* — Laboratory reagent grade.

**4.2.2.1** *Chloroform*

**4.2.2.2** *Methanol*

**4.2.2.3** *n-Hexane*

**4.2.2.4** *Diethyl ether*

**4.2.2.5** *Acetic acid*

**4.2.2.6** *Formic acid*

**4.2.3** *Oil Samples*

**4.2.3.1** *Raw Karanja oil*

**4.2.3.2** *Alkali-refined and bleached Karanja oil* — Raw *Karanja* oil is refined with 15 percent aqueous sodium hydroxide solution ( 5 percent excess over the theoretical requirement ) by stirring mechanically for 30 min at room temperature, and filtered. Refined oil is bleached with 3 percent bleaching earth and 1 percent active carbon at 120°C for 15 minutes with stirring and filtered.

**4.2.3.3** *Acid-refined and bleached Karanja oil* — Raw *Karanja* oil is stirred mechanically for 30 minutes at 40°C with 1 percent concentrated sulphuric acid and filtered. The refined oil is bleached as in **4.2.3.2**.

**4.2.3.4** *Alcoholic alkali-refined and bleached Karanja oil* — Raw *Karanja* oil is refined with 0·3 percent ethanolic ( 95 percent ) sodium hydroxide solution ( oil : alcoholic alkali, 1 : 1 ) for 45 minutes at room temperature with mechanical stirring. The oil layer is separated. The above process is repeated with 0·1, 0·1 and 0·3 percent alcoholic sodium hydroxide in three stages. After filtration, the refined oil is bleached as in **4.2.3.2**.

**4.2.3.5** *Alcohol-refined Karanja oil* — Raw *Karanja* oil is stirred mechanically for 45 minutes with 95 percent ethanol ( oil : alcohol, 1 : 3 ) at room temperature. The oil layer is separated and the process repeated once again. The refined oil is bleached as in **4.2.3.2**.

**4.2.3.6** Other oils used for preparing artificial mixtures are genuine raw oils.

### 4.3 TLC on Micro Slide

**4.3.1** *Apparatus*

**4.3.1.1** *Micro slides*

**4.3.1.2** *Coupling jar or a suitable chamber* — for developing micro slides.

**4.3.1.3** *Graduated micro pipettes or syringes* — 10 $\mu$l and 100 $\mu$l capacity.

**4.3.1.4** *Ultraviolet lamp* ( 366 nm maximum emission ).

**4.3.1.5** *Stoppered conical flask* — 250-ml.

**4.3.1.6** *Glass tube* — ( 9×4 cm ) or a suitable size beaker.

**4.3.1.7** *Drying oven*

4.3.1.8 *Desiccator*

4.3.1.9 *Sample bottles*

4.3.2 *Preparation of Micro Slides* — Take 40 g silica gel 'G' in a stoppered 250 ml conical flask and add 100 ml of a mixture of chloroform-methanol ( 7 : 3 *v/v* ). Mix the contents well by shaking and transfer the slurry into a glass tube ( 9×4 cm ). Coat the micro slides by dipping two slides held together and taking them out immediately. Dry the coated micro slides for 15 minutes at room temperature and for 15 minutes at 110°C in a drying oven. Store them in an empty desiccator.

4.3.3 *Preparation of Oil Samples* — Prepare in sample bottles 1 : 1 ( *v/v* ) solutions in chloroform of the following:

a) Oil to be tested, and

b) Pure reference oil.

4.3.4 *TLC Procedure* — Spot 1·5µl chloroform solutions of the oil to be tested and of the pure reference oil on the coated micro slide using a micro syringe or a micro pipette. Develop the micro slide with solvent mixture ( 2·5 ml ) of *n*-hexane — diethyl ether — acetic or formic acid ( 60 : 40 : 1 ) in a developing chamber until the solvent front reaches a distance of about 6 cm which takes about 7 min. Evaporate the solvent from the slide at room temperature for 5 minutes and observe in ultraviolet light. A fluorescent spot, having $R_f$ value of about 0·27, indicates the presence of *Karanja* oil in the oil under test.

4.3.5 *Detection Limit*

4.3.5.1 *Raw Karanja oil*

a) 0·1 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal or kavathi ( maroti ) oil.

b) 0·2 percent in rice bran, niger or *neem* oil.

4.3.5.2 *Alkali-refined and bleached Karanja oil*

a) 0·25 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal, kavathi, or rice bran oil.

b) 0·5 percent in niger or *neem* oil.

4.3.5.3 *Acid or alcohol or alcoholic alkali-refined and bleached Karanja oil* — 2·0 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal, kavathi, rice bran, niger or *neem* oil.

## 4.4 Regular TLC on Glass Plate

4.4.1 *Apparatus*

4.4.1.1 *Thin layer chromatographic applicator*

4.4.1.2 *Glass plates* — ( 20×20 cm, or 10×20 cm, or 5×20 cm ).

4.4.1.3 *Developing chamber*

4.4.1.4 *Drying rack*

4.4.1.5 *Graduated micro pipettes or syringes*— 10 µl and 100 µl capacity.

4.4.1.6 *Ultraviolet lamp* —( 366·nm maximum emission ).

4.4.1.7 *Stoppered conical flask* — 500-ml capacity.

4.4.1.8 *Drying oven*

4.4.1.9 *Sample bottles*

4.4.1.10 *Desiccator*

4.4.2 *Preparation of TLC Plates* — Take 120 g silica gel 'G' in a 500-ml conical flask and at 240 ml distilled water. Mix the contents well by shaking. Coat the glass plates ( 20×20 cm, 10 Nos ) using the TLC applicator adjusted to give 500 µm thick layer. Dry the coated plates at room temperature for 30 minutes, arrange on the drying rack and dry at 110°C in a drying oven for 1 h. Store them in an empty desiccator.

4.4.3 *Preparation of Oil Samples* — Prepare in sample bottles 1 : 1 ( *v/v* ) solutions in chloroform of the following:

a) Oil to be tested, and

b) Pure reference oil.

4.4.4 *TLC Procedure* — Spot 10 µl chloroform solution of the oil to be tested and of the pure reference oil on the coated plate using a micro syringe or a micro pipette. Develop the plate with a solvent mixture ( 100 ml ) of *n*-hexane-diethyl ether—acetic or formic acid (70 : 30 : 1 ) in a developing chamber until the solvent front reaches a distance of about 18 cm which takes about 55 minutes. Evaporate off the solvent from the plate at room temperature for 10 minutes and observe in ultraviolet light. A fluorescent spot, having a $R_f$ value of about 0·27, indicates the presence of *Karanja* oil in the oil under test.

4.4.5 *Detection Limit*

4.4.5.1 *Raw Karanja oil*

a) 0.05 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal or kavathi oil.

b) 0·1 percent in rice bran, niger or *neem* oil.

**4.4.5.2** *Alkali-refined and bleached Karanja oil*

a) 0·1 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal or kavathi oil.

b) 0·25 percent in rice bran or *neem* oil.

c) 0·5 percent in niger oil.

**4.4.5.3** *Acid or alcoholic alkali-refined and bleached Karanja oil*

a) 0·5 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal, kavathi or rice bran oil.

b) 2·0 percent in niger or *neem* oil.

**4.4.5.4** *Alcohol-refined and bleached Karanja oil*

a) 1·0 percent in groundnut, soybean, safflower, mustard, coconut, sunflower, sesame, palm, cottonseed, linseed, castor, ambadi, mahua, thumba, dhupa, sal or kavathi oil.

b) 2·0 percent in rice bran, niger or *neem* oil.

**4.5 Remarks**—Another fluorescent spot, having $R_f$ value of about 0.39 is also visible faintly with adulterated oils of groundnut, safflower, mustard, coconut, sunflower, sesame, cottonseed, linseed, ambadi, thumba and sal and clearly with adulterated oils of mahua, kavathi, niger and *neem*. Though less sensitive than TLC on regular plate, TLC on micro slide is more convenient, quicker and suitable for routine analysis. Regular TLC on glass plate is more useful for confirmative analysis.

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